HERITABLE VARIATIONS AND THE RESULTS OF SELECTION IN THE FISSION RATE OF STYLONYCHIA PUSTULATA

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AUSTIN RALPH MIDDLETON

Dissertation Submitted to the Board of University Studies
of the Johns Hopkins University in Conformity
with the Requirements for the Degree of
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From the Zoölogical Laboratory of the Johns Hopkins University

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INTRODUCTION

Organisms multiplying without admixture of two parents that differ in hereditary constitution have been found remarkably constant in their inherited characteristics. Most recent work agrees that in such uniparental reproduction inherited variations occur rarely or not at all, and that selection has practically no effect in altering racial characteristics. These results have given origin to the concept of the Genotype (Johannsen), as a designation for the permanent heritable constitution of the race. In view of the importance of these relations for the problem of the method of evolution, much further study of this matter is required. The present paper deals with the inheritance of varia-

tions and the effect of selection in the case of a most delicately poised and readily modifiable physiological character, the rate of fission of an infusorian multiplying without conjugation.

Most studies of the fission rate in infusoria have consisted in observations on the rate in given species, for descriptive purposes; or in examinations of the direct effect of changed physical and chemical conditions; or in the study of the effect of conjugation on the rate of multiplication. Review of these studies is not demanded here.

A certain number of observations have been made which bear on the inheritance of the fission rate and on the question whether there are differences among the progeny of a single individual in respect to these. The great papers of Maupas ('88 and '89) contain some of the most important of these. Maupas made extensive studies of the effects of temperature and of other conditions on the fission rate in many species of infusoria; among the rest in Stylonychia pustulata, the subject of the present study. Maupas became convinced, as a result of his studies, that under given conditions the rate of fission is uniform in all the progeny of a given individual; that inherited variations in the rate do not occur in such a 'pure race' or to use the modern term, within a single clone. On this matter the conclusions of Maupas are in complete harmony with those of the 'pure line' workers and upholders of the constancy of the genotype, in more recent times. He gives detailed observations on the matter for a number of species, and resumes his results as follows:

> In all my cultures I have always seen all the normal descendants of the same ancestor, grow and multiply with the most perfect uniformity. I have become convinced of the integral transmission of the faculty of development from one generation to another, and the most complete physiological equivalence must exist among all the normal individuals, produced in the successive generations ('88, p. 203).
>
> In long and numerous experiments on fifteen to twenty species, I have never observed anything which permits belief in the existence of morphological and physiological differences, not merely between the two products of a given fission, but even among all those which have descended from such a fission by regular and continuous generations ('88, p. 176).

The paper of Jennings on the Effect of Conjugation in Paramecium ('13) likewise deals to a certain extent with this matter. In a wild population many strains differing in rate of fission (under the same conditions) were found to occur. Furthermore, it was demonstrated that even in a population derived by fission from a single individual (that is, in a 'pure strain'), conjugation produced inherited differences in the fission rate, so that after conjugation there were present strains showing constant differences in these respects.

On the other hand, if no conjugation has occurred among the progeny of a single individual, the fission rate was found to be nearly or quite uniform. Jennings sums up as follows:

> It is found (1) that differences in rate of fission among those that have not conjugated since they were derived from a single parent are not inherited (unless possibly certain differences of a minimal character are to be excepted; differences of an order of magnitude far below those with which we are dealing); (2) that conjugation among the members of such a pure race does result in differentiations that are inherited ('13, p. 366).

The paper of Calkins and Gregory ('13) on the other hand sets forth that there are in many cases differences in the fission rate among the four sets of progeny resulting from the first two divisions of an individual that has just conjugated.

Other papers bearing less directly on this matter will be taken up in the discussion of the results of the present work.

The specific problem

When a single infusorian divides, often one of its two progeny again divides before the other does. In successive generations this same thing may be repeated. Thus, as shown in figure 1, one may have among the progeny of a single individual at a given moment, animals that are the products of four and others that are the products of two fissions. Hence there are differences of fission rate among the descendants of a single individual—differences that afford the opportunity of selection with a frequency that made it appear worth while to determine whether these differences are inherited and whether slow lines

and fast lines can, by selection, be isolated among the descendants of a single parent. I have carried out this work for Stylonychia pustulata. The animals were kept isolated and transferred daily to fresh culture medium; for the fast lines the individuals that divide first are uniformly selected; for the set to be developed into lines having a slow rate of fission the individuals that divide last are taken.

No attempts have ever been made heretofore to test the effect on the fission rate of selection among the progeny of a single individual. It appeared possible, though hardly probable, that such a physiological character might give results differing from those obtained from studies of the mainly structural characters hitherto examined. The work was undertaken at the suggestion of Prof. H. S. Jennings, to whom my sincere thanks are due for assistance throughout the work.

The fundamental questions for examination are then as follows: Can we, with respect to the character examined, get from a single genotype by selection two genotypes that differ characteristically from each other under identical conditions; and that retain these differences from generation to generation? Is selection of small variations, such as appear within the pure strain or clone an effective evolutionary procedure?

TECHNIQUE

In any investigation of a physiological character which is so delicately responsive to all environmental changes as is the fission rate of infusoria, the statement of Calkins ('02, p. 141)—"A correct method is the sine qua non of successful experiments with Protozoa;"—applies with peculiar force. In order that results may be of any value conditions must be uniform throughout.

Jennings ('13) has pointed out that to secure this uniformity the bacterial content must not vary. In addition to uniformity the technique used in work on the fission rate must insure the experimenter against the introduction of a 'wild' individual into the cultures and against the contamination of the lines inter se. These results have been secured by the adoption of the follow-

ing method, which is a modification of that described by Jennings ('13):

As culture medium, $\frac{1}{16}$ of 1 per cent Horlick's malted milk was employed, a fresh supply being made daily. This is the medium adopted by Miss Peebles ('12). One gram of the malted milk powder was dissolved in a 100 cc. graduate in a few cc. of boiling spring water; this was then diluted to 100 cc. with more of the boiling spring water. Six and one-quarter cc. of this 1 per cent solution were next diluted to 100 cc. with boiling spring water and this $\frac{1}{16}$ per cent solution was filtered and cooled.

The animals were cultivated on ground glass slides having each two circular depressions capable of holding four or five drops of liquid apiece. These were kept in moist chambers. Three drops of culture medium were used in each depression and no cover-glasses were employed. The 'fast' lines were kept in the left concavities and the corresponding slow lines in the right concavities of the same slides, so that conditions were uniform for the two sets.

Uniformity of bacterial content in the culture medium was secured by washing the animals in fresh culture medium before transferring to a new slide. The animals were allowed to swim about for a time in the fresh medium, in order to wash themselves largely free of bacteria; they were then transferred to the definitive slide, in new fluid. The pipette used in transferring the individuals was invariably sterilized in boiling water after each transfer, thus absolutely preventing the unintentional introduction of any individual which might cling to the pipette; there was thus no possibility of admixture of the 'fast' and 'slow' sets. The slides were labeled in lead pencil; the number of fissions and selections at each examination were likewise recorded on them, to be later transferred to permanent records. The individual lines were designated in accordance with the plan set forth by Jennings ('13).

Each concavity contained characteristically two parent individuals, the products of a single fission. The slides were examined daily or oftener. When one of the two individuals was

found to be divided, while the other was not, a selection was made (fig. 1). If the line is under selection for the production of a rapid rate of fission, the two progeny of the individual that has divided are transferred to the new slide, while the undivided individual is rejected. In selection for a slow rate of fission, on the other hand, the individual not yet divided is selected for

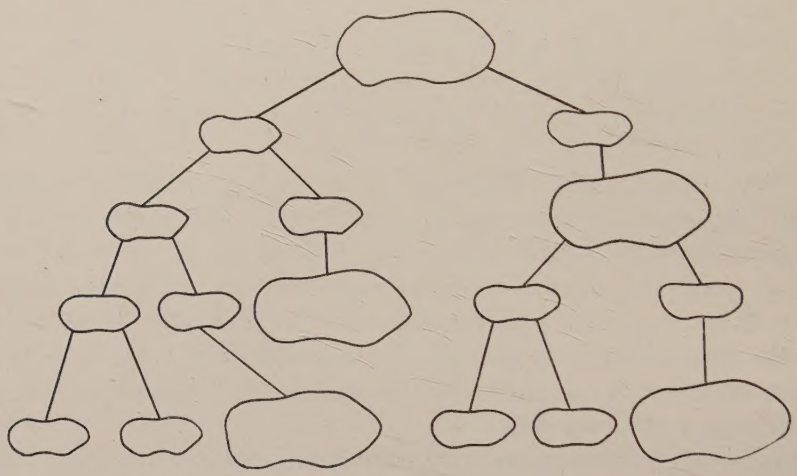

Fig. 1 Diagram of successive fissions among the progeny of a single individual, illustrating the variations in fission rate which were the subject of selection in the present work. The left side of the figure traces a series of 'fast' selections, the right side a series of 'slow' selections, showing that at a given moment we may have, among the progeny of a single parent individuals of the fourth and of the second generation.

propagating the stock. This process is continued: a 'selection' is made and counted whenever one of the two parents is found to have thus divided before the other.

EXPERIMENTS TO TEST THE EFFECT OF SELECTION ON THE FISSION RATE WITHIN A SINGLE CLONE

1. The first series of experiments

Experiment 1, part 1. Direct selection in opposite directions, November 3 to December 3, 1913.

On November 3, sixty animals of the sixth generation from a single individual which had been isolated from a 'wild' labora-

tory culture of Stylonychia pustulata were isolated on ground glass slides, one animal to each concavity, thus dividing the sixty animals into two groups of thirty each; one group to be selected for a fast fission rate ('plus selection'), the other for a slow one ('minus selection'). For twenty-one days these thirty 'fast' and thirty 'slow' lines were propagated with frequent selection as described above. On the twenty-second day the fast and the slow lines were duplicated (by division) and on the twenty-third day the first forty of the resulting sixty fast lines and the corresponding slow lines were all duplicated so that now we had one hundred fast lines and one hundred slow lines, plus and minus selection continuing with all these. These were thus propagated until the end of the third ten-day period. The actual number of fissions per line and the actual number of selections that were made during the three ten-day periods are shown for the first thirty lines of each set in juxtaposition in table 1.

When the differences per ten days of the sixty lines are averaged it shows that on the average the 'fast' lines have produced 2.03 generations more than the average for the 'slow' lines during the first ten-day period, 3.57 generations more in the second ten-day period and 2.40 generations more in the third ten-day period. Further, the fast lines have each averaged 2.67 generations more per ten days, during the whole thirty days, than the slow lines. In other words, this table shows that on the average each of these thirty fast lines has produced 0.267 generation more per day during this thirty-day period than has each slow line. Figure 2 is the curve of the difference between the daily averages of the two sets.

It is clear that the direct effect of the selections made would be to produce a difference in favor of the 'fast' lines as long as plus and minus selection continues, even though the differences were not hereditary and were due purely to accidental causes. Our next test is therefore to determine whether any hereditary result has been produced; to do this, selection must cease, and we must determine whether the differences in rate still continue. The most obvious method would be to stop selecting and pick

TABLE 1

Experiment 1, Part 1: Number of fissions (generations) and number of selections per line per ten-day period, for the first thirty lines of both the fast and slow sets while opposite selection was in progress; with the excess in favor of the fast set. (Differences given the minus sign show an excess of the slow set)

Lines Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	TOTAL	AVERAGE PER LINE
First 10-day Period:																																
Fast:																																
Selections.....	3	2	2	3	2	4	3	3	4	5	3	2	3	3	4	1	2	4	3	4	4	4	4	7	5	5	5	5	3	2		
Generations.....	19	20	18	19	19	22	22	22	20	17	20	19	17	19	20	19	19	19	19	20	23	19	20	21	21	21	20	20	23	16	593	19.76
Slow:																																
Selections.....	2	4	1	3	2	5	2	2	4	7	7	4	3	4	6	4	4	3	5	4	3	2	5	6	3	2	4	4	7	4		
Generations.....	18	17	18	16	18	18	16	19	18	17	15	19	16	15	17	19	15	19	18	16	18	17	20	18	20	19	18	18	19	21	532	17.73
Excess in favor of 'fast'.....	1	3	0	3	1	4	6	3	2	0	5	0	1	4	3	0	4	0	1	4	5	2	0	3	1	2	2	2	4	−5	61	2.03
Second 10-day Period:																																
Fast:																																
Selections.....	3	6	4	5	5	5	5	5	4	5	4	4	6	5	6	7	5	5	5	4	5	4	5	6	6	5	5	5	6	6		
Generations.....	21	17	17	18	18	21	18	21	21	19	20	20	18	18	17	20	17	16	17	18	24	21	21	19	18	19	23	18	17	18	570	19.00
Slow:																																
Selections.....	5	5	2	7	4	4	4	3	6	5	7	5	6	2	4	4	6	7	3	3	6	7	7	7	5	4	5	3	2	4		
Generations.....	17	16	13	14	13	15	16	17	15	15	17	16	17	16	16	15	15	14	16	13	17	18	15	15	16	15	15	16	15	15	463	15.43
Excess in favor of 'fast'.....	4	1	4	4	5	6	2	4	6	4	3	4	1	2	1	5	2	2	1	5	7	3	6	4	2	4	8	2	2	3	107	3.57
Third 10-day Period:																																
Fast:																																
Selections.....	1	5	5	8	4	4	5	4	4	3	5	6	5	4	6	4	5	3	6	5	6	5	6	4	5	6	6	7	6	2		
Generations.....	23	20	20	20	20	21	24	22	20	22	25	23	23	24	23	22	21	21	22	22	25	23	24	22	23	25	25	25	26	25	681	22.70
Slow:																																
Selections.....	2	5	5	8	4	4	5	5	6	5	7	5	4	5	6	4	2	6	7	6	4	4	5	7	7	3	6	3	4	7		
Generations.....	21	18	17	15	18	20	18	19	17	19	22	23	20	20	20	21	20	20	19	19	23	23	20	20	21	23	23	22	24	24	609	20.30
Excess in favor of 'fast'.....	2	2	3	5	2	1	6	3	3	3	3	0	3	4	3	1	1	1	3	3	2	0	4	2	2	2	2	3	2	1	72	2.40
Fast:																																
Total Selections....	7	13	11	16	11	13	13	12	12	13	12	12	14	12	16	12	12	12	14	13	15	13	15	17	16	16	16	17	15	10		
Total Generations..	63	57	55	57	57	64	64	65	61	58	65	62	58	61	60	61	57	56	58	60	72	63	65	62	62	65	68	63	66	59	1844	61.46
Slow:																																
Total Selections....	9	14	8	18	10	13	11	10	16	17	21	14	13	11	16	12	12	16	15	13	13	13	17	20	15	9	15	10	13	15		
Total Generations..	56	51	48	45	49	53	50	55	50	51	54	58	53	51	53	55	50	53	53	48	58	58	55	53	57	57	56	56	58	60	1604	53.46
Excess in favor of 'fast'.....	7	6	7	12	8	11	14	10	11	7	11	4	5	10	7	6	7	3	5	12	14	5	10	9	5	8	12	7	8	−1	240	8.00

at random the animals for daily transfer to the fresh slides. But this method opened the possibility of the 'personal equation' becoming so important a factor that I rejected it. In place of it I devised what I have called the method of 'balanced selection.' Balanced selection is the process of compensating for the effect of every selection that one is compelled to make by making the reverse selection at the next opportunity; in other words, one makes the same number of plus and minus selections in any given line during each successive time interval adopted. Thus, if on a given day one makes a plus selection, at the next oppor-

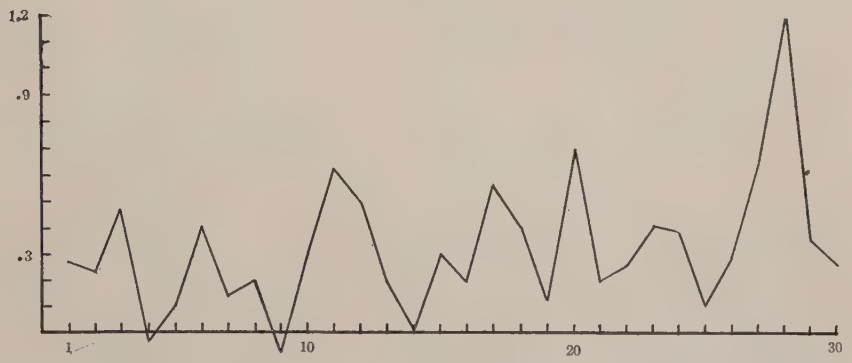

Fig. 2 Curve of the daily differences between the average number of generations per line produced by each of the two sets of lines while under selection in opposite directions (Exp. 1, part 1). The ordinates show the average differences (in fractions of a generation) in favor of the fast selected lines; the abscissae are the days.

tunity he would make a reverse, or minus selection. In order to be able to tell at a glance whether to make a plus or minus selection the character of the selection to be made was also recorded on the slide.

In addition to carrying out balanced selection, in a portion of the lines reversed selection was practiced; that is, the fast lines were now subjected to minus selection, the slow lines to plus selection. Of course, if the observed difference in rate is hereditary and has resulted from selection, then there is no logical reason why the same result should not be obtained a second time, so that the difference would disappear, or eventually be

replaced by a reversed difference. The experiment with balanced and reversed selection is that described next.

Experiment 1-A Balanced selection and reversed selection, December 4 to December 23, 1913.

This experiment was undertaken to test by the methods set forth above whether the difference in fission rate shown at the end of thirty days of opposite selection by the two sets of lines in Experiment 1, part 1, were hereditary. In the first place fifty of the slow lines and fifty of the fast lines were subjected

TABLE 2

Experiment 1-A: Actual number of generations per ten lines per ten-day period for the 50 fast and 50 slow lines of Experiment 1, Part 1, when subjected to balanced selection

NUMBER OF LINES	11 TO 20	21 TO 30	31 TO 40	71 TO 80	91 TO 100	AVERAGE PER LINE PER 10-DAY PERIOD	AVERAGE PER LINE PER DAY
First 10-day Period:							
Fast lines	152	160	161	160	143	15.52	1.552
Slow lines	155	158	167	148	136	15.28	1.528
Differences in favor of the fast lines	−3	2	−6	12	7	0.24	0.024
Second 10-day Period:							
Fast lines	90	95	88	99	100	9.44	0.944
Slow lines	88	89	87	97	86	8.94	0.894
Differences in favor of the fast lines	2	6	1	2	14	0.50	0.050

to balanced selection for twenty days. Table 2 gives the actual number of generations that these fifty fast and slow lines produced. The sets are divided into groups of ten lines each.

The average difference of fission rate per line per day for the whole thirty days of Experiment 1, part 1, while selection was in progress was 0.267 generation in favor of the fast lines. For the twenty days of balanced selection it was 0.037 generation. This would seem to indicate that the direct selection of Experiment 1, part 1, had produced a very slight heritable difference in fission rate between the two sets, especially since the difference 0.05 generation of the second ten-day period of balanced

selection is considerably larger than for the first, 0.024 generation; and further it suggests the conclusion that if direct selection were practiced long enough a greater difference might be established.

TABLE 3

Experiment 1-B: Actual number of generations per ten lines per ten-day period for the fifty fast lines and fifty slow lines subjected to reversed selection ('fast' lines selected now for slow fission, 'slow' lines for fast fission)

NUMBER OF LINES	1 TO 10	41 TO 50	51 TO 60	61 TO 70	81 TO 90	AVERAGE PER LINE PER 10-DAY PERIOD	AVERAGE PER LINE PER DAY
First 10-day Period:							
Slow lines, *plus* selected:							
Selections	29	42	47	55	48		
Generations	157	187	174	166	180	17.28	1.73
Fast lines, *minus* selected:							
Selections	36	42	35	39	35		
Generations	147	160	150	137	145	14.78	1.48
Difference in favor of lines now plus selected	10	27	24	29	35	2.50	0.25
Second 10-day Period:							
Slow lines, *plus* selected:							
Selections	24	28	23	38	25		
Generations	96	108	88	110	96	9.96	1.00
Fast lines, *minus* selected:							
Selections	22	21	17	34	33		
Generations	95	93	92	93	95	9.36	0.94
Difference in favor of lines now plus selected	1	15	−4	17	1	0.60	0.06

Reversed selection. Table 3 gives the actual number of fissions of the fifty fast and slow lines under reversed selection. Again the lines of each set are divided into groups of ten each.

Table 3 shows that reversed selection has made the average fission rate of the fifty slow lines faster than the average fission rate of the fifty fast lines. Now, as pointed out above, if the observed difference in fission rate is hereditary and has resulted from selection this result might logically be expected to follow from reversed selection. The net result of both phases of this

Experiment 1-A, while favorable to the production of an hereditary effect through selection, was to show that opposite selection must continue for a much longer period before the nature of the result can be established beyond controversy.

Experiment 1, part 2. Continued opposite selection, December 4 to December 23, 1913.

While Experiment 1-A was in progress certain fast and slow lines of Experiment 1, part 1, were kept under direct selection as a precaution against the possibility that selection in that experiment had not yet produced heritable differences in the fission rate. For this purpose fast lines 6, 30, 43, 45, 60, 73, 76, 78 and 100 and slow lines 3, 4, 9, 41, 53, 71 and 85 were chosen. During the first of these two ten-day periods the animals were transferred to fresh slides every twenty-four hours and during the second ten-day period, every forty-eight hours. The actual number of generations produced by each of these lines, during the two ten-day periods is shown in table 4.

TABLE 4

Experiment 1, Part 2: Actual number of generations and selections per line per ten-day period, with the excess of generations in favor of the 'fast.' Direct selection of the fourth and fifth ten-day periods of Experiment 1.

LINES NUMBER — FAST / SLOW	6 / 3	30 / 4	43 / 9	45 / 41	60 / 53	73 / 71	76 / 85	78	100	AVERAGES
								omitted in averages		
Fourth 10-day Period:										
Fast:										
Selections	3	6	4	4	4	4	7	2	3	
Generations	14	16	18	18	17	20	17	18	18	17.14
Slow:										
Selections	2	5	3	6	5	3	5			
Generations	12	11	13	16	18	15	18			14.71
Excess in favor of 'fast'	2	5	5	2	−1	5	−1			2.43
Fifth 10-day Period:										
Fast:										
Selections	1	3	1	3	3	3	2	3	2	
Generations	9	10	9	10	10	8	9	9	9	9.28
Slow:										
Selections	2	2	2	2	2	2	3			
Generations	7	4	7	5	4	5	8			5.71
Excess in favor of 'fast'	2	6	2	2	6	3	1			3.57

This experiment is simply a connecting link between part one and part three of Experiment 1. There is a slight increase in the difference between the average number of generations produced by the fast and slow lines during the first and second ten-day periods. The fourth and fifth ten-day periods of figure

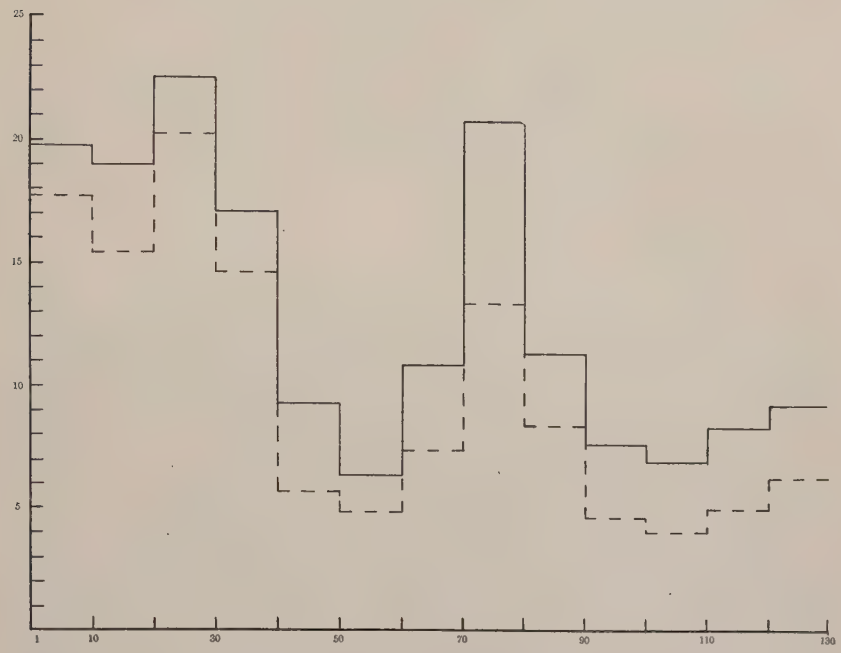

Fig. 3 Polygon of the average number of generations produced, per line, by the fast and slow selected sets of lines of Experiment 1, while the two were undergoing selection in opposite directions; averaged for ten-day periods. The polygon shows this average for each of all the 13 consecutive ten-day periods of Experiment 1. The continuous line shows the average for the fast lines, the broken one the average for the slow lines. The ordinates give the average number of generations produced and the abscissae the number of days since the beginning of the experiment—the successive ten-day periods.

3 show the average number of generations per line per ten-day period for this part of the first experiment and its first three ten-day periods show it for the first part; while figure 4 is the curve of the daily differences between the average number of generations produced by each fast and each slow line during these two ten-day periods.

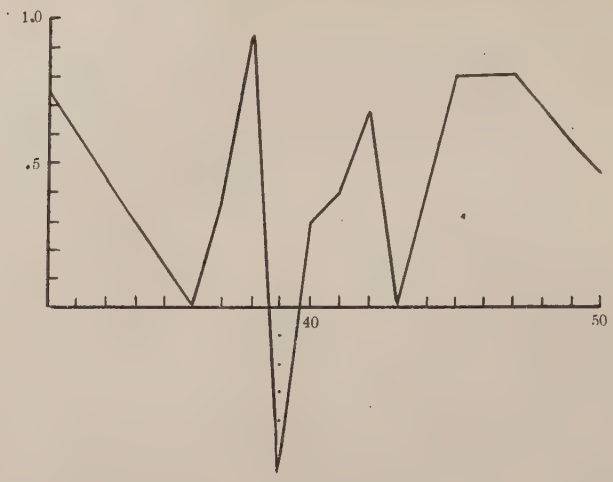

Fig. 4 Curve of the daily differences between the average number of generations per line produced by the fast selected set and the slow selected set during the fourth and fifth ten-day periods of opposite selection in Experiment 1, (i.e., Exp. 1, part 2.) The ordinates give the daily differences, the abscissae give the days.

Experiment 1, part 3. Continued opposite selection, December 24, 1913, to January 22, 1914.

It will be noticed from table 1 and figure 3 that the differences between the average number of generations produced by the fast lines and the slow lines during the three consecutive ten-day periods of Experiment 1, part 1, are 2.03, 3.57 and 2.40. That is, each fast line produced, on the average, 0.267 generation more per day than each slow line during these thirty days. Table 4 and figure 3 show that the corresponding differences for the fourth and fifth ten-day periods of Experiment 1, were 2.43 and 3.57 which gives a daily average difference of 0.300 generation per line. This difference, in favor of the average fast line is slightly (0.033 generation), greater than the corresponding difference of Experiment 1, part 1 (0.267 generation). Does this mean that on the average, selection is gradually increasing this difference? Experiment 1-A indicates that opposite selection had produced a heritable difference in the average fission rate of the two sets of lines; may this heritable difference be increased by further opposite selection?

To answer this question the first seven of the fast lines of Experiment 1, part 2, were each increased (by division) to four and from the eighth two lines were derived, giving thirty fast lines in all. Each of the slow lines of Experiment 1, part 2, was likewise increased to four, with the exception of the last one which was increased to six, thus giving thirty slow lines. These two sets of lines were then selected for fast and slow rates of fission. During the three ten-day periods of this part of Experiment 1, the selection and transfer of animals to fresh slides was made every forty-eight hours instead of at the close of every twenty-four hour interval as heretofore.

At the end of the first ten-day period the eight fast lines which had produced the highest number of generations were selected and thirty lines were derived from them, care being taken that each group of ten lines, the 'fast' occupants of a single moist chamber, were represented in the new set. Also eight of the slow lines which had produced the smallest number of generations were selected and from them thirty lines for continued slow selection were derived. The same precaution was taken in reference to the distribution of the selected lines among the three moist chambers. These two sets of lines were then selected for fast and slow fission rates during the second ten-day period. Finally this same method of reduplication of the fastest and the slowest lines and the subsequent continued selection of the individual variations of fission rate was followed through the third ten-day period.

This method of double selection was devised with the purpose of crowding as much selection into the experiment as possible, in the attempt to determine whether continued selection would gradually increase the previously established difference between the average rates of fission of the two sets of lines. It will be remembered that the difference of average fission rates between the two sets of lines at the end of Experiment 1, part 2, was 3.57 generations per line per ten-day period as shown by the fifth ten-day period of figure 3 and by table 4. The sixth seventh and eighth ten-day periods of figure 3 show those differ' ences for the three ten-day periods of the present part of Experi-

ment 1. For the first period of this part (sixth period of the entire experiment) the difference was 1.53 generations per line per ten-day period, for the next it was 3.41 generations and for the last it was 7.51 generations. The small difference of 1.53 between fast and slow in period 6, following upon a period when the difference was 3.57, is due to a slowing of the fission rate in all lines during period 6, owing probably to low temperature, and perhaps partly also to one of the rhythms emphasized by Woodruff and his colleagues. The percentage of difference in proportion to the total average fission rate is in reality greater in period 6 than in preceding periods. Thus, for period 4 the average number of fissions for all sets was 15.925, and the difference between the fast and slow was 2.43—this difference being thus 15.25 per cent of the average rate. In period 6, the small difference 1.53 is actually 27.34 per cent of the average rate for all. Table 5 gives the actual number of generations produced by each of the lines during the three consecutive ten-day periods, the number of selections that were made in each line and the average difference per line for each ten-day period.

Table 5 and figure 3 demonstrate in this experiment a continued increase of the difference between the average rates of fission of the two sets of lines. As has each of the previous experiments, so also does this one show that the fission rate within the clone may be changed by selection. During its three ten-day periods each fast line produced on the average 0.415 generations more per day than each slow one. On only one day during the thirty days of this experiment did the slow lines produce more generations than the fast lines and on that day only an average of 0.3 generation per line, as shown by figure 5. The two sets indeed hardly overlap at all in their rates, practically all of the fast set being faster than any of the slow set. This is shown by the curves of variation of the two sets in figure 6. Only one slow line produced as many generations as the slowest fast line.

TABLE 5

Experiment 1, Part 3: Actual number of generations and of selections per ten-day period per line of the third part of Experiment 1, that is, the sixth, seventh and eighth ten-day periods of continuous opposite selection, with the excess in generations produced in favor of the fast-selected set

LINES NUMBER	1 TO 10	11 TO 20	21 TO 30	TOTAL	AVERAGE PER LINE PER TEN-DAYS
Sixth 10-day Period:					
Fast:					
Selections	13	14	14		
Generations	62	65	64	191	6.36
Slow:					
Selections	5	8	11		
Generations	44	48	53	145	4.83
Excess in generations in favor of the fast	18	17	11	46	1.53
Seventh 10-day Period:					
Fast:					
Selections	28	22	21		
Generations	121	101	104	326	10.87
Slow:					
Selections	23	25	23		
Generations	69	88	67	224	7.46
Excess in generations in favor of the fast	52	13	37	102	3.41
Eighth 10-day Period:					
Fast:					
Selections	32	42	41		
Generations	168	222	236	626	20.87
Slow:					
Selections	31	31	36		
Generations	132	133	134	399	13.30
Excess in generations in favor of the fast	36	89	102	227	7.56
Fast:					
Total for 30 days	351	388	404	1143	38.10
Slow:					
Total for 30 days	247	271	254	772	25.73
Difference	104	117	150	371	12.37

Experiment 1-B. Balanced selection, January 23 to April 22, 1914.

At the close of Experiment 1, part 3, the two sets of lines of that experiment had been under continuous opposite selection

for eighty days, and the average difference per line per day had increased from 0.267 generation for the first thirty days to 0.415 generation for the last thirty days of that period. To test the permanence of this apparent effect of selection, to determine the answer of our experiments to the question "Can we get from a single genotype by selection two genotypes that differ charac-

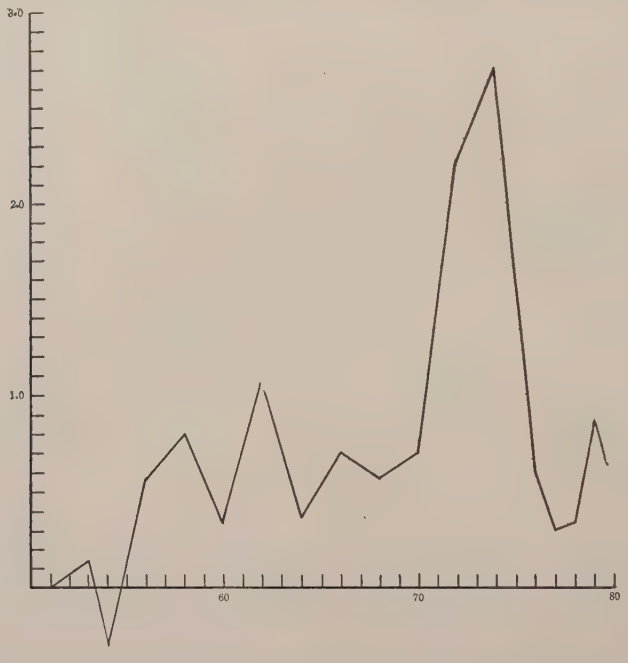

Fig. 5 Curve of the daily difference between the average number of generations per line produced by the fast set and the slow set during the sixth, seventh and eighth ten-day periods of opposite selection in Experiment 1 (Exp. 1, part 3). The ordinates give the daily excess in favor of the fast-selected lines, the abscissae give the days.

teristically from each other under identical conditions; and that retain these differences from generation to generation?"—the lines were now subjected to the test of balanced selection. For this purpose all the thirty fast and thirty slow lines which were in progress at the close of Experiment 1, part 3, were continued. In order to make the test thorough it was prolonged through nine consecutive ten-day periods, or ten-days longer than the

lines had been subjected to opposite selection. During this whole experiment the animals were transferred to fresh slides daily as described at the beginning of this paper.

The results of Experiment 1-B, are so important that they are set forth in some detail in table 6. This table gives the actual number of generations produced by each fast and each

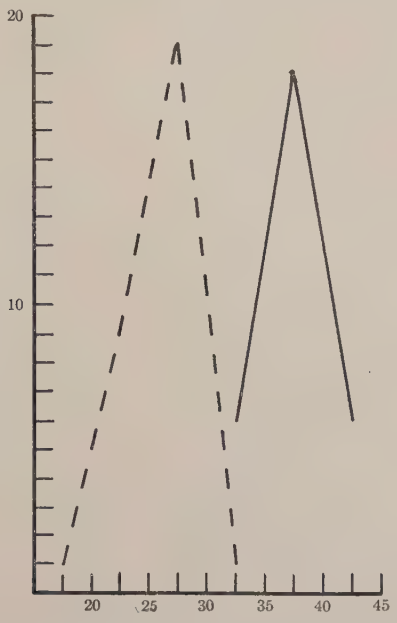

Fig. 6 Curves of variation in actual number of fissions of the two sets of lines of Experiment 1, Part 3. The ordinates give the number of lines, the abscissae the number of generations produced during the thirty days. The continuous line is the curve of the fast-selected set, the broken one is that of the slow-selected set.

slow line during each ten-day period of the experiment, and the difference between them. This difference is given as a positive quantity when the fast line has produced more generations than the corresponding slow one and negative when the reverse is the case. The latter occurred only thirty-one times among the whole two hundred and seventy differences. In twelve of the lines it did not occur at all; in nine lines, once; in six lines, twice;

TABLE 6

Experiment 1-B: Actual number of generations per ten-day period per line of Experiment 1, parts 1, 2 and 3, for the nine consecutive ten-day periods of balanced selection which immediately followed the eighty days of direct selection of these parts of Experiment 1. The differences in number of fissions in favor of the fast lines are given also.

LINES NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	TOTAL	AVERAGE	PER CENT THAT THE DIFFERENCE IS OF THE TOTAL IN ALL
First 10-day Period:																																	
Fast, Generations	17	21	15	18	15	16	19	17	13	12	14	17	13	9	9	12	14	11	11	11	12	9	12	13	10	13	12	14	11	12	402	13.40	
Slow, Generations	15	12	16	15	14	11	14	11	11	13	12	8	7	9	10	12	6	9	8	8	11	9	8	9	6	8	9	9	9	9	308	10.26	
Difference.....	2	9	−1	3	1	5	5	6	2	−1	2	9	6	0	−1	0	8	2	3	3	1	0	4	4	4	5	3	5	2	3	94	3.14	13.27
Second 10-day Period:																																	
Fast, Generations	8	7	8	6	8	6	6	7	8	6	7	3	8	4	7	9	6	7	6	5	7	7	8	8	5	5	6	5	8	6	197	6.57	
Slow, Generations	8	8	5	6	5	4	6	5	5	5	7	5	5	5	6	5	5	3	5	5	5	5	4	6	2	3	3	5	5	1	147	4.90	
Difference.....	0	−1	3	0	3	2	0	2	3	1	0	−2	3	−1	1	4	1	4	1	0	2	2	4	2	3	2	3	0	3	5	50	1.67	14.55
Third 10-day Period:																																	
Fast, Generations	10	10	8	7	8	8	10	12	8	6	8	8	6	7	6	8	6	6	6	6	9	8	8	8	9	10	8	8	8	7	237	7.90	
Slow, Generations	8	9	8	7	6	10	8	8	6	8	7	8	5	7	6	8	4	4	7	8	6	4	5	6	5	6	3	5	4	4	190	6.33	
Difference.....	2	1	0	0	2	−2	2	4	2	−2	1	0	1	0	0	0	2	2	−1	−2	3	4	3	2	4	4	5	3	4	3	47	1.57	11.03
Fourth 10-day Period:																																	
Fast, Generations	9	12	10	9	13	11	9	10	11	12	9	9	10	6	8	6	7	8	7	8	8	6	9	7	9	10	10	9	9	11	272	9.06	
Slow, Generations	10	10	7	10	8	11	7	6	11	8	8	4	5	7	6	10	5	7	9	7	5	4	5	6	8	5	7	5	6	9	216	7.02	
Difference.....	−1	2	3	−1	5	0	2	4	0	4	1	5	5	−1	2	−4	2	1	−2	1	3	2	4	1	1	5	3	4	3	2	56	1.86	11.43
Fifth 10-day Period:																																	
Fast, Generations	11	12	15	9	13	15	15	13	15	15	12	10	12	7	12	9	5	9	9	10	11	8	11	13	7	9	10	9	9	14	329	10.96	
Slow, Generations	12	12	4	8	10	14	9	4	12	12	7	8	6	4	8	7	8	7	7	7	6	5	5	5	8	7	12	8	7	11	240	8.00	
Difference.....	−1	0	11	1	3	1	6	9	3	3	5	2	6	3	4	2	−3	2	2	3	5	3	6	8	−1	2	−2	1	2	3	89	2.96	15.61
Sixth 10-day Period:																																	
Fast, Generations	14	11	15	11	10	14	16	16	16	15	12	9	9	9	14	11	8	11	8	12	10	8	13	13	13	9	8	10	10	14	349	11.63	
Slow, Generations	14	12	12	10	12	11	13	9	11	11	10	6	6	9	13	11	8	6	6	8	3	6	0	9	9	5	11	6	4	12	263	8.77	
Difference.....	0	−1	3	1	−2	3	3	7	5	4	2	3	3	0	1	0	0	5	2	4	7	2	13	4	4	4	−3	4	6	2	86	2.86	14.01

Seventh 10-day Period:																															
Fast, Generations	11	11	13	16	12	14	13	11	11	11	9	9	13	10	12	13	11	10	9	14	7	7	7	12	10	12	11	10	11	11	331	11.03	
Slow, Generations	9	10	10	12	13	14	12	5	8	12	8	10	9	10	10	8	7	7	7	5	4	4	6	7	7	6	9	4	8	6	247	8.23	
Difference.....	2	1	3	4	−1	0	1	6	3	−1	1	−1	4	0	2	5	4	3	2	9	3	3	1	5	3	6	2	6	3	5	84	2.80	14.53
Eighth 10-day Period:																															
Fast, Generations	11	12	13	13	14	15	15	14	13	14	11	13	13	13	14	11	11	12	11	12	8	12	11	12	11	14	10	13	10	12	368	12.26	
Slow, Generations	8	11	10	9	14	16	15	5	11	13	11	14	13	10	13	11	10	10	5	3	4	6	8	6	9	7	9	7	2	11	281	9.36	
Difference.....	3	1	3	4	0	−1	0	9	2	1	0	−1	0	3	1	0	1	2	6	9	4	6	3	6	2	7	1	6	8	1	87	2.90	13.41
Ninth 10-day Period:																															
Fast, Generations	13	13	14	17	18	17	14	14	8	10	13	13	12	8	10	12	11	9	9	13	10	8	9	10	10	13	11	11	7	11	348	11.60	
Slow, Generations	7	8	10	14	11	13	12	12	12	14	11	12	14	12	6	9	6	5	7	8	6	5	8	6	5	7	8	4	5	6	268	8.76	
Difference.....	6	5	4	3	7	4	2	2	−4	−4	2	2	−2	−4	4	3	5	4	2	5	4	3	1	4	5	6	3	7	2	5	85	2.83	13.89
Differences for the whole 90-days....	13	17	29	15	18	12	21	49	16	5	4	16	26	0	14	10	20	25	15	32	32	25	39	36	25	41	15	36	33	29	678	22.83	

In this table the numbers in the 'difference' columns when positive indicate that the fast line in question has produced so many more generations than the corresponding slow one; thus during the first ten-day period fast line No. 1, produced 2 more generations than slow line No. 1. When the number in the difference column is negative the slow line has produced more generations than the corresponding fast one during the ten-day period; this is the case for slow line No. 10 during the first ten-day period.

The average difference in favor of the fast, per ten-day period during this experiment, is 2.51 generation per line.

in two lines, three times; and in one line four times. When the differences between the number of generations produced by the corresponding lines during the whole ninety days were ascertained it was found that in *not a single case had the slow line produced more generations than its fast one.* And in only two lines was the excess of the fast over the slow probably too small to be significant (fast line ten produced only five more generations than slow line ten, and the two lines number fourteen produced the same number of generations). Furthermore during each ten-day period the *total number of generations produced by all the thirty fast lines was much larger than that produced by the slow lines, and the average number of generations per line per ten-day period was uniformly greater for the fast than for the slow lines.* Also the per cent of the difference in proportion to the total number of generations produced by both sets was calculated and found to be remarkably uniform; these percentages are shown in the extreme right hand column of table 6. Finally, the average number of generations per line per ten-day period for each set of lines is plotted as a polygon in figure 7-*a*, giving a graphic representation of that phase of table 6.

Figure 8 gives the curve of the daily differences between the average number of generations produced by each fast and each slow line. On only three days during the whole ninety days of this balanced selection experiment was this difference too small to be significant (on the eighth and sixteenth of February, 1914, both sets of lines produced the same number of generations and on February 18, 1914, the difference was 0.03 in favor of the slow set). The average per day for each fast line, including the three instances just cited, was 0.251 generation greater than each slow line. From table 6 and from figures 7 and 8 it is therefore evident that, when measured by the test of balanced selection, the eighty days of opposite selection had produced a difference of fission rate between the two sets of lines that is heritable. Furthermore, analysis of the daily records shows that this result is not due to the chance isolation of a 'mutating' line in either set of lines and the subsequent development of the thirty lines of

the set from that one, for we have four lines of the fast set that run all the way through and three of the slow lines that do the same. Table 7 shows the total number of generations produced by each line of both sets. For the fast set these totals vary from

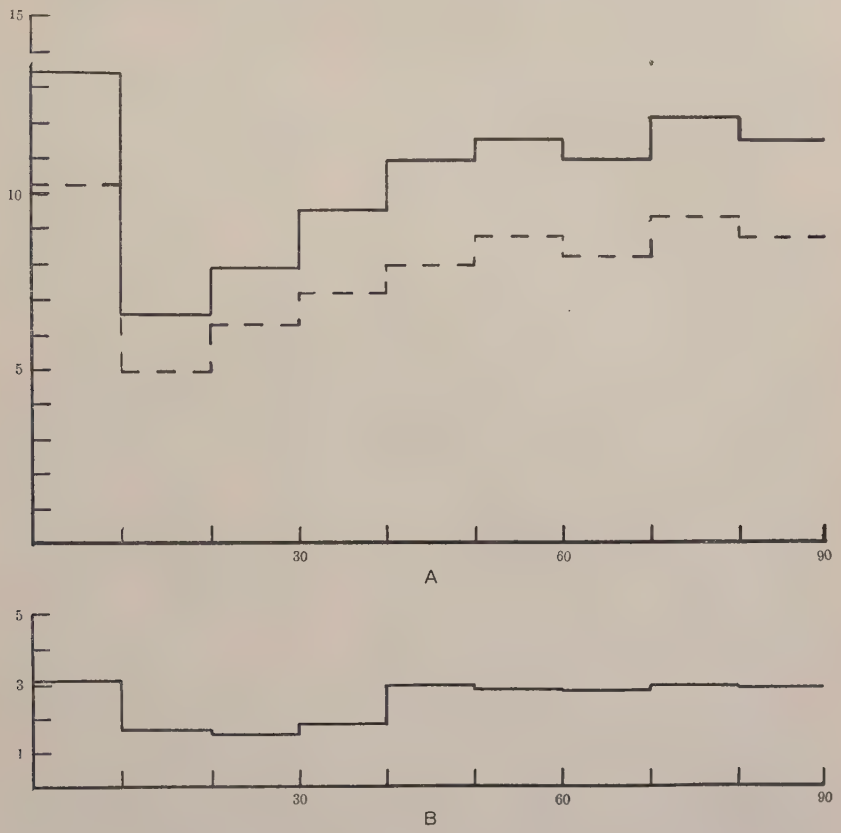

Fig. 7-*a* Polygons of the average number of generations per line per ten-day period produced by the fast and slow sets of lines of Experiment 1-B during its nine consecutive ten-day periods of balanced selection. The continuous line shows the averages for the fast set, the broken line the averages for the slow set. The ordinates show the differences of the averages, the abscissae the ten-day periods.

Fig. 7-*b* Curve of the difference between the average number of generations per line per ten-day period produced by the fast and slow sets of lines of Experiment 1-B during its nine consecutive ten-day periods of balanced selection. The ordinates show the differences of the averages in favor of the fast set, the abscissae the consecutive ten-day periods.

TABLE 7

Experiment 1-B: The total number of generations produced by each fast and slow line during balanced selection for the ninety days of the experiment, with the difference between the nvmber of generations produced by each fast line and its corresponding slow line. Also the sum of the number of generations produced by each fast line and its corresponding slow line, and the percent that the differences are of the sums

LINES NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Fast, number of generations in 90 days	104	109	111	106	111	116	117	114	103	101	95	91	96	73	92	91	79	83	76	91	82	73	88	96	84	95	86	89	83	98
Slow, number of generations in 90 days	91	92	92	91	93	104	96	65	87	96	81	75	70	73	78	81	59	68	61	59	50	48	49	60	59	54	71	53	50	69
Totals	195	201	193	197	204	220	213	179	190	197	176	166	166	146	170	172	138	141	137	150	132	121	137	156	143	149	157	142	133	164
Excess in favor of fast	13	17	29	15	18	12	21	49	16	5	14	16	26	0	14	10	20	25	15	32	32	25	39	36	25	41	15	36	33	29
Per cent that excess is of total	6.66	8.45	15.02	7.61	8.82	5.45	9.85	27.37	8.42	2.53	7.95	9.63	15.66		8.23	5.81	14.38	17.73	10.94	21.33	24.24	20.66	28.46	23.07	17.48	27.51	9.55	25.35	24.81	17.68

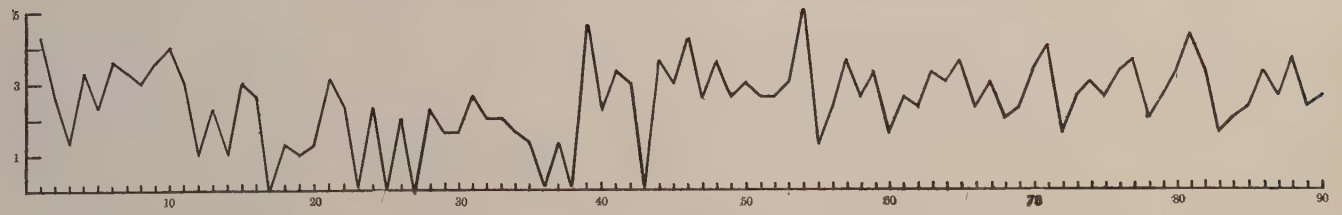

Fig. 8 Curve of the daily difference between the average number of generations per line produced by the fast and slow sets of lines of Experiment 1-B during its ninety days of balanced selection. The ordinates show the differences of the daily averages in favor of the fast set; the abscissae are the ninety days. The ordinates of this curve, averaged for ten-day periods, give figure 7-*b*.

seventy-three to one hundred and seventeen and for the slow set from forty-eight to ninety-six with the exception of one line which produced one hundred and four generations. The first ten lines of both sets each produced a much larger number of generations than the remainder of the lines of its own set. These lines were the occupants of a single moist chamber, so that there was probably some environmental difference distinguishing this moist chamber from the other two. That environmental differences have a marked effect on the fission rate of infusoria has of course been shown by many writers.

Figure 9-*a* gives the curves of variation in number of generations produced by each fast and each slow line during balanced selection. The curve for the slow lines shows an apparent bimodality. To determine whether this might be attributable to the environmental difference suggested by table 7 these same variation curves were plotted for the first ten lines of each set alone. These curves are shown as figure 9-*b*; the curves are mutually exclusive. Table 7 shows that only one slow line among these ten produced more generations than the slowest one of these first ten fast lines. Figure 9-*c* gives the curves of variation for fast and slow lines 11 to 30, the occupants of the other two moist chambers. Only four of these twenty slow lines produced more generations during the ninety days of balanced selection than the slowest fast line.

It must be borne in mind in this connection that these "two sets of thirty lines" are parts of the same clone. That is, we have got here, from a single genotype by selection two genotypes that differ characteristically from each other under identical conditions; and that retain these differences from generation to generation.

Experiment 1, part 4. Further opposite selection, January 23 to March 14, 1914.

While Experiment 1-B was in progress the original lines were continued under direct selection for two reasons: 1) As a precaution against the possible failure of the difference of fission

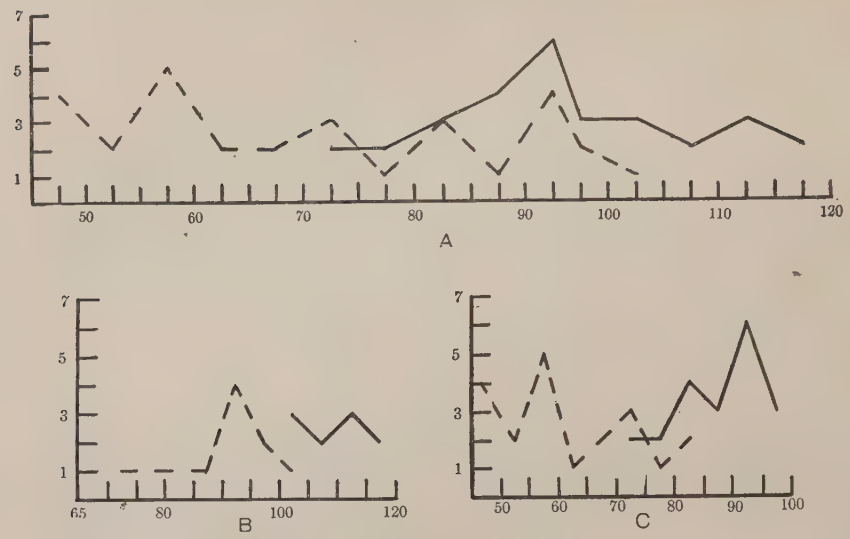

Fig. 9-*a*. Curves of variation of the lines of Experiment 1-B, the ninety-days of balanced selection. The ordinates give the number of lines, the abscissae the number of generations produced. The continuous line is the curve of the fast set, the broken line that of the slow set.

Fig. 9-*b* Curves of variation of the first ten fast and first ten slow lines of Experiment 1-B (balanced selection). The ordinates represent numbers of lines, the abscissae, generations. The continuous line is the curve of the fast set, the broken one the curve of the slow set.

Fig. 9-*c* Curves of variation of the last twenty fast and last twenty slow lines of Experiment 1-B (balanced selection). The ordinates represent numbers of lines, the abscissae, generations. The continuous line is the curve of the fast set, the broken one the curve of the slow set.

rate to survive long continued balanced selection (Experiment 1-B); 2) To discover what would be the effect if selection continued further on these lines. During the first three ten-day periods there was no further reduplication of extreme lines and the slides were changed daily. At the end of the third ten-day period the fastest lines of the fast set and the slowest lines of the slow set were again chosen for reduplication and continued selection, as described for Experiment 1, part 3. This was the only time this was done during the present experiment. The slides were changed every forty-eight hours during the fourth and fifth ten-day periods. The ninth to the thirteenth ten-day

periods of figure 3 (which gives the average fission rates of the two sets of lines of the entire first experiment for the one hundred and thirty days they were selected), give the average fission rates of the lines of this part of Experiment 1. Table 8 gives the actual number of generations per 30 lines as well as the average number of selections and generations per line that occurred and the differences in the average fission rates.

It has been pointed out that, on the average, for the first three ten-day periods of Experiment 1 each fast line produced

TABLE 8

Experiment 1, Part 4: Actual and average number of generations and of selections per 30 lines per ten-day period during the ninth to the thirteenth ten-day periods of continuous opposite selection of the lines of Experiment 1

TEN-DAY PERIODS	NINTH	TENTH	ELEVENTH	TWELFTH	THIRTEENTH	TOTAL	AVERAGE PER LINE
Fast lines:							
Average number of selections per line.	2.57	2.47	32.3	1.63	2.30		
Total number of generations	341	230	206	250	279	1306	43.53
Average number of generations per line	11.37	7.67	6.87	8.33	9.30		
Slow lines:							
Average number of selections per line.	1.57	2.33	1.36	1.33	1.97		
Total number of generations	251	139	121	149	189	849	28.30
Average number of generations per line	8.37	4.63	4.03	4.96	6.30		
Actual excess of generations in favor of fast lines.....	90	91	85	101	90	457	15.23
Average excess per line in favor of fast lines.....	3.00	3.04	2.84	3.37	3.00		3.05

0.267 generation more per day than each slow line. During the fourth and fifth ten-day periods each fast line produced, on the average 0.300 generation more per day than each slow line. During the sixth, seventh and eighth ten-day periods this daily average difference per line was 0.415 generation. Table 8 shows that during the ninth, tenth, eleventh, twelfth and thirteenth ten-day periods it was 0.305 generation, which is considerably smaller than the 0.415 generation difference of the sixth, seventh and eighth ten-day periods of Experiment 1. But, as in a previous case, this is due merely to the fact that the average fission rate for all lines has decreased; relative to this average fission rate the difference between fast and slow lines has not decreased, but on the contrary has increased. For part 1 of Experiment 1, the difference between the fast and the slow lines in number of generations produced was 6.9 per cent of the total number of generations produced by all; for part 2 it was 12.8 per cent; for part 3, 19.3 per cent, and for part 4 it was 21.2 per cent. Consequently, throughout the entire period of selection (thirteen ten-day periods), the proportional difference between fast and slow lines has steadily increased.

Figure 10 further emphasizes the genuineness of the difference of fission rate between these two sets of lines for Experiment 1, part 4. It shows the curves of variation of these two sets of lines of that experiment.

Experiment 1-C. Mass culture and balanced selection, March 17 to May 4, 1914.

In order to demonstrate as conclusively as possible whether the apparent average difference of fission rate between the two sets of lines was hereditary or not, it was decided to subject them to a period of mass culture and then to balanced selection. This was to determine whether the average difference of fission rate had survived the mass culture treatment. On March 4, 1914, all the animals remaining in the 'fast' concavities after the transfer of the chosen individuals had been made to fresh slides were placed, unwashed in a single circular glass dish $3\frac{3}{4}$ inches in diameter and 2 inches deep in 50 cc. of $\frac{1}{32}$ per cent

Horlick's malted milk; and all those remaining in the 'slow' concavities were similarly treated. These two mass cultures were placed side by side on the laboratory table and allowed to propagate for twelve days. Every three days 25 cc. of boiled and cooled spring water was added to each to compensate for evaporation. The animals placed in these mass cultures were taken from Experiment 1-B, and hence had been subjected to opposite selection for eighty days and balanced selection for

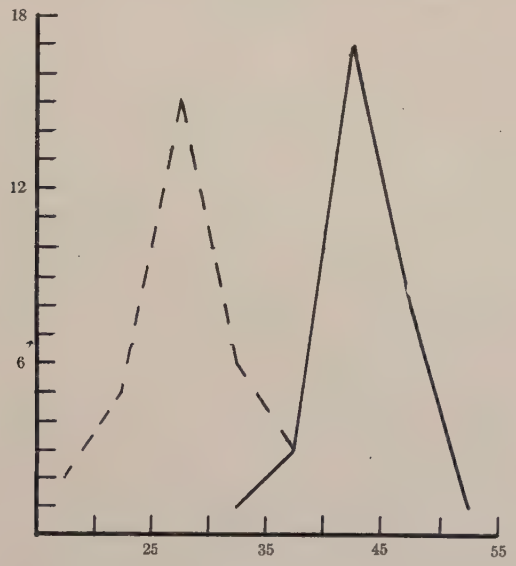

Fig. 10 Curves of variation of the lines of Experiment 1, Part 4 (continuous selection). The ordinates give numbers of lines, the abscissae the generations produced during the fifty days of the experiment. The continuous line is the curve of variation of the fast set, the broken one the curve of the slow set.

forty days; they were now allowed to remain in mass cultures for twelve days.

On March 17, 1914, thirty individuals were taken from the mass culture of fast lines and isolated on slides and thirty individuals were also isolated from the mass culture of slow lines. These sixty lines were then subjected to balanced selection for a period of fifty days, the transfer to fresh slides being made daily.

In order to be sure of the uniformity of the bacterial content of the slides, on March 18, an equal small quantity of the liquid from each of the mass cultures was added to the fresh medium. It was added two or three drops at a time and each few drops carefully studied in a watch glass under the binocular before it was added to the fresh culture medium. On March 20, each animal transferred to the fresh slides was washed in a watch glass in a mixture of equal quantities of the culture medium from the mass cultures instead of being washed in fresh culture medium.

Now at the end of this fifty-day period of balanced selection the two sets had experienced *eighty* days of *opposite selection* immediately followed by *one hundred and two days* of *no selection*. If the difference remains after this test it is evident that *selection has in this case produced an heritable difference in fission rate within the clone*. Table 9 shows that this difference of average fission rate does persist. Figure 11 shows the rate of division of the two sets of lines, averaged for ten-day periods, during this balanced selection test.

Experiment 1-D. Reversed selection, April 13 to June 1, 1914. A second experiment in reversed selection was started on April 13, 1914, from lines derived from Experiment 1-B, the ninety-day balanced-selection experiment described above. Hence they had been subjected to eighty days of opposite selection and this was followed by eighty days of balanced selection before reversed selection was started. This experiment was prolonged through five ten-day periods with daily transfer to fresh slides and during the whole time the average fission rate of the fast-selected 'slow' lines was higher than that of the slow-selected 'fast' lines. And on the whole there was a gradual increase of this average difference as reversed selection proceeded. Here again selection has altered the fission rate and the new rates were hereditary. Figure 12 and table 10 show graphically the results of this experiment.

Attention is called in table 10 to the large average number of selections per line for the fifty days of this reversed selection.

TABLE 9

Experiment 1-C: Actual number of generations per ten-day period per line. Balanced selection after mass culture

LINES NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	TOTAL	AVERAGE
First 10-day Period:																																
Fast, Generations....	12	12	12	12	14	14	15	12	12	13	13	13	10	13	13	13	12	12	13	11	14	11	10	10	11	11	11	11	12	11	363−6	11.9
Slow, Generations....	8	10	13	9	11	9	13	14	10	10	10	9	10	12	14	7	13	9	12	10	8	12	11	10	11	9	12	11	10	11	318+6	10.8
Excess in favor of fast lines.....	4	2	−1	3	3	5	2	−2	2	3	3	4	0	1	−1	6	−1	3	1	1	6	−1	−1	0	0	2	−1	0	2	0		1.1
Second 10-day Period:																																
Fast, Generations....	10	13	10	12	12	12	12	11	11	9	10	11	11	10	10	11	9	10	10	10	9	8	4	9	11	12	9	11	10	10	307−4	10.10
Slow, Generations...	8	10	9	8	9	9	8	12	3	3	7	7	7	10	11	7	9	7	6	9	6	7	8	9	10	3	10	12	7	7	238−6	7.73
Excess in favor of fast lines.....	4	3	1	4	3	3	4	−1	8	6	3	4	4	0	−1	4	0	3	4	1	3	1	−4	0	1	9	−1	−1	3	3		2.37
Third 10-day Period:																																
Fast, Generations....	7	12	13	12	11	14	14	11	9	12	12	11	11	12	10	12	11	12	9	10	12	11	7	11	11	12	11	11	11	9	330+3	11.1
Slow, Generations....	16	12	12	10	10	11	8	9	4	7	10	6	9	11	11	8	12	9	13	11	12	11	8	8	10	1	12	11	7	6	285−3	9.4
Excess in favor of fast lines.....	−9	0	1	2	1	3	6	2	5	5	2	5	2	1	−1	4	−1	3	−4	−1	0	0	−1	3	1	11	−1	0	4	3		1.7
Fourth 10-day Period:																																
Fast, Generations....	11	13	11	9	9	14	13	11	11	8	12	11	13	14	10	15	13	9	8	10	9	12	8	13	13	13	10	8	10	9	329+7	11.20
Slow, Generations....	14	13	14	11	10	11	7	12	4	5	11	11	9	9	9	12	12	5	7	12	12	12	3	11	11	2	12	9	6	9	285−5	9.33
Excess in favor of fast lines.....	−3	0	−3	−2	−1	3	6	−1	7	3	1	0	4	5	1	3	1	4	1	−2	−3	0	5	2	2	11	−2	−1	4	0		1.87
Fifth 10-day Period:																																
Fast, Generations....	16	10	15	16	13	15	17	11	9	15	14	14	13	14	12	11	12	13	12	9	11	12	12	14	15	14	13	14	11	10	387+5	13.06
Slow, Generations....	14	8	13	13	12	14	7	11	10	11	11	12	14	12	10	11	15	12	6	15	12	13	15	12	13	13	13	10	9	14	355−3	11.73
Excess in favor of fast lines.....	2	2	2	3	1	1	10	0	−1	4	3	2	−1	2	2	0	−3	1	6	−6	−1	−1	−3	2	2	1	0	4	2	−4		1.33

The column labeled 'Surplus Selections' indicates that for instance during the first 10-day period, six more fast selections were made than slow selections in the fast lines. Therefore six is subtracted from the total number (363) of generations produced and the average 11.9 is gotten by dividing this remainder (357) by 30, the number of lines.

It is 13.74 selections per line for the fast-selected 'slow' lines and 12.79 selections for the slow-selected 'fast' lines. Now the average difference of fission rate per line per day for the eighty days of balanced selection immediately preceding the present experiment was 0.25 generation per line per day and as a result of the fifty days of reversed selection this difference was wiped out and

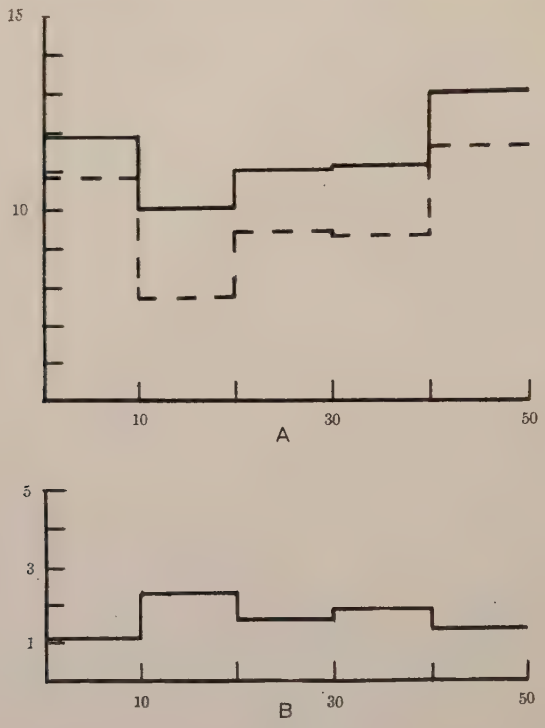

Fig. 11-*a* Polygon of the average number of generations per line per ten-day period produced by the 'fast' and 'slow' sets of lines of Experiment 1-C (balanced selection for 50 days after 80 days of selection, 40 days of balanced selection and 12 days of mass culture). The continuous line shows the averages for the 'fast' set, the broken one the averages for the 'slow' set. The ordinates are the averages per ten-day periods, the abscissae, the ten-day periods.

Fig. 11-*b* Curve of the difference in favor of the fast lines between the average number of generations per line per ten-day period produced by the 'fast' and 'slow' sets of lines of Experiment 1-C during its five consecutive ten-day periods of balanced selection after mass culture. The ordinates are the differences of the averages, the abscissae the consecutive ten-day periods.

an average difference of 0.27 generation established in the reverse direction.

If this difference shows itself to be heritable, it will appear that it is probably due to the large number of selections practised within the relatively short period of fifty days; it will indi-

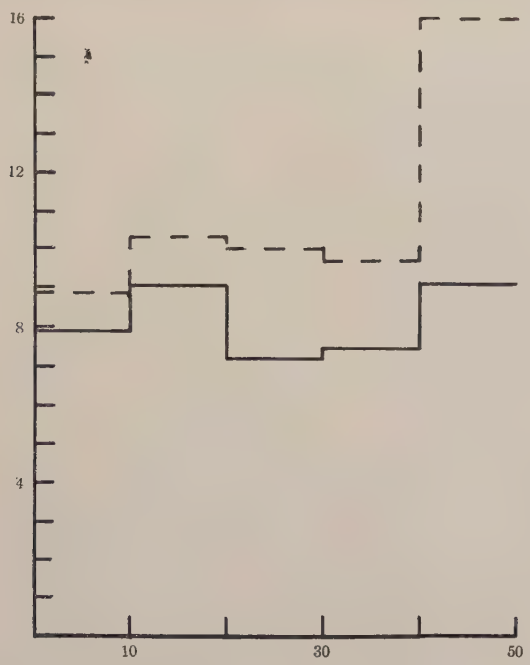

Fig. 12 Polygon of the average number of generations per line per ten-day period produced by the 'fast' and 'slow' sets of lines of Experiment 1-D (reversed selection). The continuous line of the curve of the former fast lines now slow-selected. The broken line is the curve of the former slow lines now fast-selected. The ordinates are the averages per ten-day periods, the abscissae the ten-day periods.

cate that the alteration of fission rate is proportional to the number of selections made, rather than the length of time over which the selections are distributed. This matter is tested in the next experiment.

Experiment 1-E. Balanced selection after reversed selection, June 2 to June 21, 1914.

TABLE 10

Experiment 1-D: Actual number of generations and selections per 30 lines per ten-day period during the five ten-day periods of the second reversed selection of the lines of Experiment 1

TEN-DAY PERIODS	FIRST	SECOND	THIRD	FOURTH	FIFTH	TOTAL	AVERAGE PER LINE
Fast Selected Slow Lines:							
Average number of selections	2.40	2.77	2.63	2.27	3.67		
Total number of generations	263	309	300	292	473	1637	54.57
Average number of generations	8.77	10.30	10.00	9.73	15.77		10.91
Slow Selected Fast Lines:							
Average number of selections	2.83	2.70	2.13	2.60	2.53		
Total number of generations	237	273	217	225	276	1228	40.93
Average number of generations	7.90	9.10	7.23	7.50	9.20		818
Actual excess in favor of the fast selected 'slow' lines	26	36	83	67	197	409	13.64
Average excess in favor of the fast selected 'slow' lines	0.86	1.20	2.77	2.23	6.56		2.73

The lines from the experiment in reversed selection (Experiment 1-D) just described were next continued under the balanced selection method for two ten-day periods in order to determine whether the differences in the reverse direction obtained in that experiment were true heritable differences of fission rate. The animals were transferred to fresh slides daily. Figure 13 shows the polygon of the average fission rates of these two sets of lines and table 11 gives the actual number of generations produced by each set. From these it appears that the average daily difference of fission rate per line between the two sets of lines is 0.38 generation in favor of the fast selected slow set.

It is therefore clear that reversed selection had really produced an heritable difference in fission rate in favor of the former slow lines; the relative rates of fission of the two sets was now reversed. As before remarked, this result is to be expected; the earlier experiments show that direct selection produces hereditary differences in one direction; reversed selection

in the same way produces hereditary differences in the opposite direction.

General results of the first group of experiments. The experiments thus far described have all dealt with parts of a single clone of Stylonychia pustulata; selection has been practised on

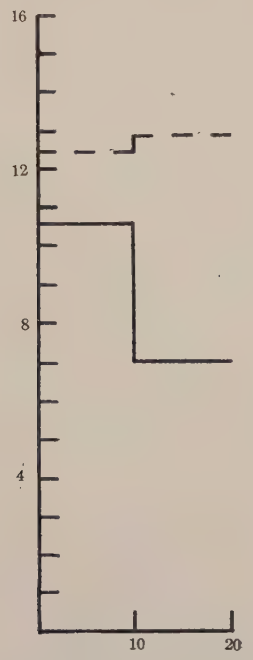

Fig. 13 Polygon of the average number of generations per line per ten-day period produced by the fast and slow sets of lines of Experiment 1-E (balanced selection after the reversed selection of Experiment 1-D). The continuous line is the curve of the reversed selected former fast lines now balanced selected. The broken line is the curve of the reversed selected former slow lines now balanced selected. The ordinates are the averages per ten-day periods; the abscissae the ten-day periods.

the variations in fission rate within the clone. For one hundred and thirty days two halves of this clone were subjected to selection in opposite directions; this produced a marked and steadily increasing difference in the average fission rate of the two halves. Expressing the excess of generations produced by the fast-selected lines as a percentage of the total number of

TABLE 11

Experiment 1-E: Actual number of generations per 10-day period per line. Balanced selection

LINE NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	TOTAL	SURPLUS SELECTION	AVERAGE
First 10-day Period:																																	
Fast	11	11	15	11	13	9	11	11	14	12	16	16	15	15	13	9	11	14	14	14	13	13	9	13	13	9	9	13	12	13	372	0	12.40
Slow	11	11	11	11	11	10	10	10	10	10	11	11	11	11	11	11	10	10	9	9	11	11	11	11	11	11	11	10	10	9	317	0	10.56
Excess in favor of the fast selected lines	0	0	4	0	2	−1	1	1	4	2	5	5	4	4	2	−2	1	4	5	5	2	2	−2	2	2	−2	−2	3	2	4			1.84
Second 10-day Period																																	
Fast	14	13	10	13	17	13	14	15	11	11	10	10	11	11	15	16	16	21	11	20	12	12	14	9	10	15	14	9	11	9	387	0	12.86
Slow	5	7	8	7	8	6	7	8	9	8	5	6	8	7	7	5	9	10	6	6	9	9	7	7	5	8	9	6	8	2	212	0	7.06
Excess in favor of the fast selected lines	9	6	2	6	9	7	7	7	2	3	5	4	3	4	8	11	7	11	5	14	3	3	7	2	5	7	5	3	3	7			5.80

TABLE 12

Experiment 1, Parts 1, 2, 3 and 4: Total number of generations produced by each line, fast and slow during the 13 10-day periods of Experiment 1

LINE NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	TOTAL
Fast	185	183	185	183	184	181	181	186	178	182	181	180	185	186	183	185	187	185	186	184	184	185	180	183	179	180	186	181	187	185	5506
Slow	124	125	127	126	128	128	127	122	124	126	125	125	122	128	125	117	123	122	123	120	123	118	119	119	118	119	120	116	119	120	3678
Excess in favor of the fast lines	61	58	58	57	56	53	54	64	54	56	56	55	63	58	58	68	64	63	63	64	61	67	61	64	61	61	66	71	68	65	1828
Range of Fast lines	178 to 187																														
Range of Slow lines	116 to 128																														

generations produced by both sets, the difference was 6.9 per cent in part 1; 12.8 per cent in part 2; 19.3 per cent in part 3, and 21.2 per cent in part 4. Table 12 summarizes the generations produced by each line of each set throughout all parts of the experiment. It shows that for the fast lines the number of generations ranges from 178 to 187, while for the slow lines the range is but from 116 to 128. Thus there is no overlapping in the two sets; the slowest fast selected line has produced 50 more generations than the fastest slow selected line.

To determine whether the difference in fission rate thus produced is heritable, parts of the two sets were removed at intervals and subjected to culture without selection ('balanced selection'). In every case it was found that the difference was heritable. Also, representatives of the two sets after eighty days of selection and forty days of no selection, were subjected to mass culture for twelve days. Further line culture for fifty days without selection showed that the inherited difference in fission rate still persisted. Thus the inherited difference produced by selection had lasted for one hundred and two days without selection.

Experiments with reversed selection showed that the inherited difference could be reversed as readily as it is produced; the originally fast set was thus caused to become the slower one, and vice versa. Continuation of these sets without selection showed again that the difference so produced was heritable.

Thus in this case the selection of small individual variations in fission rate has split the single clone (derived vegetatively from a single parent) into two hereditarily diverse divisions (diverse clones).

2. The second series of experiments

The results of the first series of experiments appeared so important and in some respects unexpected that it was felt necessary to control them by repetition, beginning again with a single individual, and endeavoring anew to procure from it by selection two hereditarily diverse sets. This was first at-

tempted with a single individual taken from one of the fast lines of the first set of experiments, giving experiment 2, described below. It was later carried out anew with the progeny of a single 'wild' individual ('third series').

Experiment 2-A. Selection among the progeny of a single individual from Experiment 1. The individual selected for repetition of the experiment was one of those belonging to a fast line of the previous experiment. The progeny of this individual did not live well, so that in some cases one or both sets died out before any definite result was obtained. In one case, however, selection of fast and slow sets was continued through nine consecutive ten-day periods (April 5 to July 3, 1914). During every ten-day period except the first set, the fast-selected set produced more generations than the slow-selected one. The results are less striking than in Experiment 1, however, in the fact that on twenty-two days out of the ninety, the slow lines produced more generations than the fast ones. The irregularity appears connected with the high mortality in both sets. However, the average difference in fission rate per line per day in favor of the fast selected set was 0.317 for the first thirty days, 0.757 for the second thirty days; and 0.61 for the third thirty days.

Experiment 2-B. Now the two sets resulting from the ninety days' selection in Experiment 2-A were subjected to balanced selection for ten days. The difference in fission rate persisted to the extent of an average difference in favor of the fast lines of 0.28 generation per line per day, though on one day the slow lines were faster by 0.01 generation per line. Before the end of the next period all lines were dead, owing perhaps to the hot weather.

Results. The evidence from these two experiments is, so far as it goes, in the same direction as from those of the first set. Selection produced from the progeny of a single individual two sets differing hereditarily in average fission rate.

3. The third series of experiments

Experiment 3-A. September 22 to October 21, 1914.

To further test the results thus far reached, and to determine whether they are based on conditions generally occurring in the organism studied, a new wild individual was obtained from a new mass culture brought into the laboratory September 15, 1914. From this, two sets of thirty individuals each, all belonging to the seventh filial generation, were obtained, and sub-

TABLE 13

Experiment 3-A: Actual number of generations and of selections per ten-day period per thirty lines of the 30 fast and 30 slow lines isolated among the progeny of the single 'wild' individual subjected to opposite selections for 30 days

TEN-DAY PERIODS	FIRST	SECOND	THIRD	TOTAL	AVERAGE PER LINE
Fast Lines:					
Average number of selections.	2.53	1.60	1.50		
Total number of generations.	973	634	603	2210	73.66
Average number of generations	32.43	21.13	20.10		
Slow Lines:					
Average number of selections.	2.90	1.53	0.96		
Total number of generations.	935	581	523	2039	67.96
Average number of generations	31.16	19.36	17.43		
Actual excess in favor of the fast lines	38	53	80	171	5.70
Average excess in favor of the fast lines	1.27	1.77	2.67		1.90
Percent the difference is of the total for both	1.99%	4.36%	7.10%		

jected, in the manner previously described, one to 'fast,' the other to 'slow' selection. In this series only one individual, in place of two, was selected from each line at each change, and the selections were made daily. There was no reduplication of the fastest and slowest lines. Fourteen of the fast and twenty-one of the slow persisted intact throughout experiments 3-A and 3-B.

Opposite selection was practised for thirty days. The records are given in table 13. In all three ten-day periods the fast-selected lines produce more generations than the slow-selected,

though in four days of the first period the reverse is true. In all the other twenty-six days the average of the fast lines was above that of the slow. The table shows a gradual increase in the number of generations produced by the fast lines relative to those produced by the slow, the excess in favor of the fast

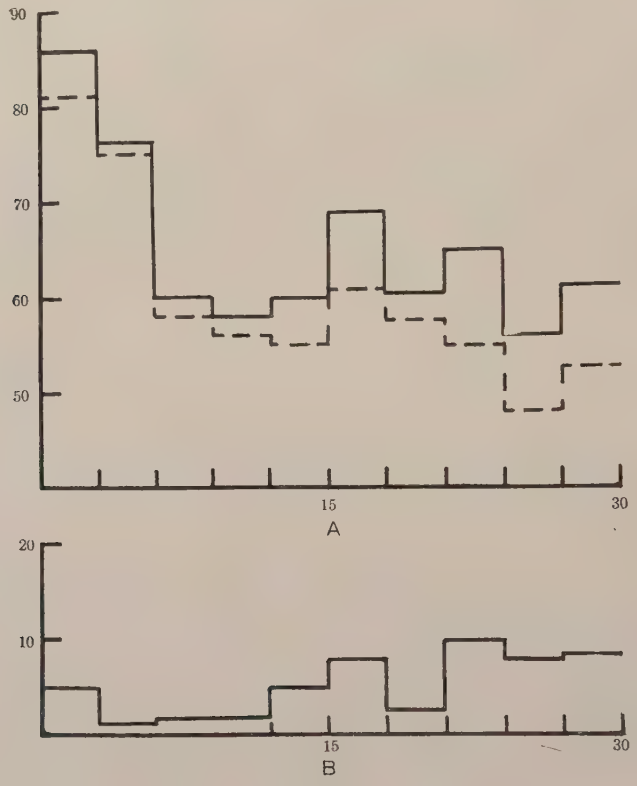

Fig. 14-*a* Polygon of the average number of generations per line per three day period produced by the fast and slow sets of lines of Experiment 3-A (direct selection in opposite directions of the progeny of the second wild individual). The continuous line is the curve of the fast set, the broken line, the curve of the slow set. The ordinates are the averages, the abscissae the three-day periods.

Fig. 14-*b* Curve of the difference (in favor of the fast lines) between the average number of generations per line per three-day period produced by the fast and slow sets of lines of Experiment 3-A (direct selection). The ordinates are the differences between the averages; the abscissae, the consecutive three-day periods. Note the progressive increase of the difference under opposite selection.

being for the three successive ten-day periods respectively 1.99 per cent, 4.36 per cent, and 7.10 per cent of the total number of generations produced in the given period.

The gradual increase of the difference between the two sets indicates that this difference was heritable. This gradual increase is well shown in the curves of figure 14, giving at *a*, the average number of generations produced per three-day period by each set, at *b* the curve of the differences in favor of the fast set. Figure 15 gives the curves of variation of the total number of generations produced by the two sets, showing that they overlap very little. The evidence indicates strongly that the effect of selection is cumulative.

Experiment 3-B. To test whether the difference produced by selection is actually heritable, balanced selection was now practiced for twenty-one days, October 21 to November 10, 1914. On every day but one (the second) the lines that had been subjected to fast-selection averaged higher than others. Table 14 gives the actual numbers of generations per line for the two ten-day periods.

Table 14 shows that during both of its periods the fast lines averaged more generations than the slow ones and further that the per cent that this difference is of the total number of generations produced by both together was practically constant. Figures 14-*c* and 14-*d* show the average difference of fission rate between these two sets of lines, averaged for three-day periods. These two figures emphasize the marked uniformity of this average difference. Hence this experiment shows that the opposite selection previously practised had produced a nearly uniform heritable difference of average fission rate between the two halves of this second clone. The results are thus the same as in our first set of experiments.

Experiment 3-C. Effects of conjugation on the results of selection.

It appeared of interest to determine whether these inherited results of selection would persist through conjugation. It is well known that conjugation is an ordeal having many effects on

TABLE 14

Experiment 3-B: Actual number of generations per ten-day period per line of the lines of Experiment 4-A for the twenty-one days of balanced selection *immediately following the thirty days of opposite selection of Experiment 4-A, with the differences in favor of the fast-selected lines. The column marked per cent shows what percentage this difference is of the total produced by both sets*

LINES NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	TOTAL	AVERAGE	PER CENT
First 10-day Period:																																	
Fast, Generations	15	19	17	18	16	17	17	15	18	18	17	17	15	15	18	18	16	17	18	18	17	18	18	16	17	16	17	18	19	17	512	17.06	
Slow, Generations	15	15	16	18	12	12	13	13	14	17	15	16	17	16	19	18	16	16	16	18	17	18	14	16	17	15	16	16	18	17	473	15.76	
Difference.....	0	4	1	0	4	5	4	2	4	1	2	1	−2	−1	3	−1	0	1	2	0	0	0	4	0	0	1	1	2	1	0	39	1.30	3.97
Second 10-day Period:																																	
Fast, Generations	24	21	20	17	20	15	22	15	16	18	19	20	18	22	22	20	20	23	25	25	22	19	23	22	24	19	20	23	22	22	618	20.60	
Slow, Generations	24	20	16	18	10	17	13	15	18	14	12	17	18	21	20	21	21	20	24	23	20	20	19	22	20	19	18	17	21	21	559	18.63	
Difference.....	0	1	4	−1	10	−2	9	0	−2	4	7	3	0	1	2	−1	−1	3	1	2	2	−1	4	0	4	0	2	6	1	1	59	1.97	5.01
Total generations fast.....	39	40	37	35	36	32	39	30	34	36	36	37	33	37	40	38	36	40	43	43	39	37	41	38	41	35	37	41	41	39	1130	37.66	
Total generations slow.....	39	35	32	36	22	29	26	28	32	31	27	33	35	37	35	40	37	36	40	41	37	38	33	38	37	34	34	33	39	38	1032	34.40	
Total generations both.....	78	75	69	71	58	61	65	58	66	67	65	70	68	74	75	78	73	76	83	84	76	75	74	76	78	69	71	74	80	77	2162	72.06	
Difference of F. & S. totals.....	0	5	5	−1	14	3	13	2	2	5	9	4	−2	0	5	−2	−1	4	3	2	2	−1	8	0	4	1	3	8	2	1	98	3.27	4.53

vitality and reproductive power; often changing the fission rate. Conjugation was obtained among the selected individuals of this third set of experiments, and its effect tested.

Watch glass cultures were made in $\frac{1}{32}$ per cent malted milk from each of the thirty fast lines and the thirty slow lines. On

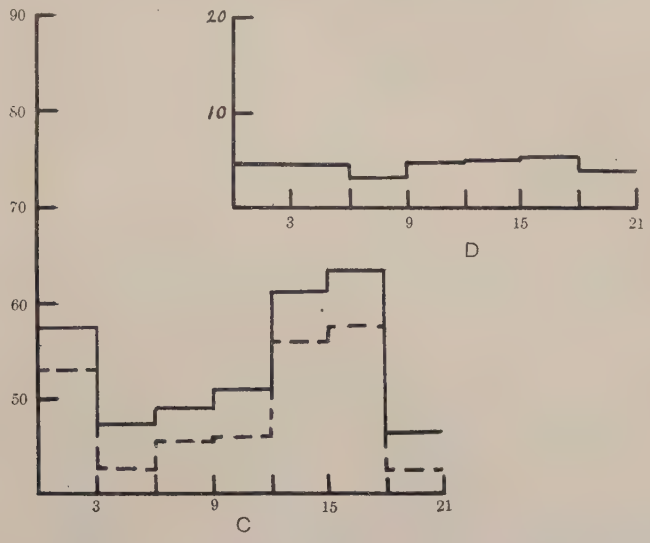

Fig. 14-*c* Polygon of the average number of generations per line per three-day period produced by the fast and slow sets of lines of Experiment 3-B (balanced selections after the direct selection of Experiment 3-A). The continuous line is the curve of the fast set, the broken line is the curve of the slow set. The ordinates are the averages, the abscissae the three-day periods.

Fig. 14-*d* Curve of the difference (in favor of the fast lines) between the average number of generations per line per three-day period produced by the fast and slow sets of lines of Experiment 3-B (balanced selection after the direct selection of Experiment 3-A). The ordinates are the differences between the averages; the abscissae, the consecutive three-day periods. Note the practical uniformity of the difference under balanced selection.

December 4 and succeeding days conjugating pairs were obtained, some from the fast lines, some from the slow ones. Though many of the ex-conjugants died, eventually representatives propagating normally were obtained from six of the fast lines and four of the slow ones of Experiment 3-B.

It will be recalled that these animals, before conjugation, had been subjected to opposite selection for thirty days, then to

twenty-one days of culture without selection and twenty-three days of mass culture. On December 7, sixty ex-conjugants were isolated from the fast lines (ten from each of six diverse lines), and sixty from the slow lines (twenty from each of two slow lines, ten from each of two others). They were cultivated in the way previously described. Balanced selection (i.e., in effect no selection) was practised for fifteen days. Table 15 gives the numbers of generations produced by the fast and slow lines during the five three-day periods of this experiment. No lines were lost by death, and practically no selection of any sort was necessary.

The daily record sheets of this experiment show that during its first five days the slow lines averaged more generations per line than did the fast lines. Table 15 shows this for the first

TABLE 15

Experiment 3-C: Actual number of generations of the 60 fast lines of ex-conjugants and of the 60 slow lines of ex-conjugants during balanced selection for 15 days. The two members of each conjugating pair were individuals of the same line

THREE-DAY PERIODS	FIRST	SECOND	THIRD	FOURTH	FIFTH	TOTAL	AVERAGE PER LINE
Fast lines:							
Total number of generations	378	302	188	237	192	1297	21.63
Average number of generations	6.30	5.03	3.13	3.95	3.20		
Slow lines:							
Total number of generations	416	310	178	225	181	1310	21.83
Average number of generations	6.93	5.16	2.96	3.75	3.02		
Actual excess in favor of the fast lines	−38	−8	10	12	11	−13	−0.20
Average excess in favor of the fast lines	−0.63	−0.13	0.17	0.20	0.18		−0.04
Per cent of the excess in favor of the fast lines in terms of the total for both	−4.78%	−1.30%	2.73%	2.60%	2.94%		

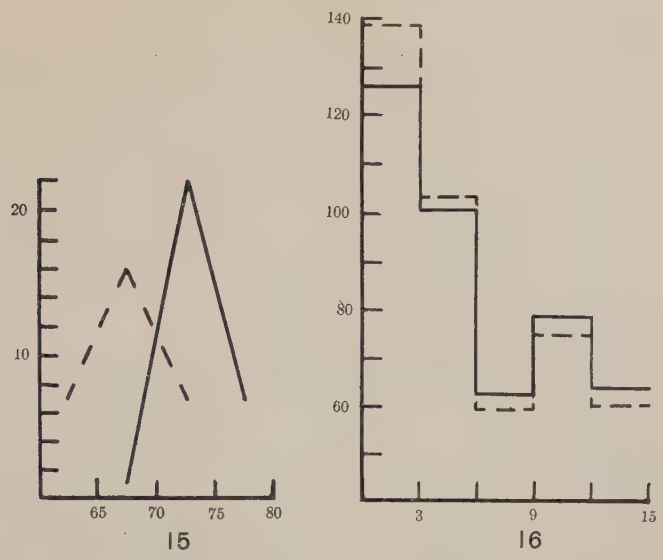

Fig. 15 Curves of variation of the lines of Experiment 3-A (direct selection of progeny of second wild individual). The ordinates give numbers of lines; the abscissae the number of generations. The continuous line is the curve of the fast set; the broken line, the curve of the slow set.

Fig. 16 Polygon of the average number of generations per line per three-day period produced by the fast and slow sets of lines of Experiment 3-C (balanced selection after conjugation). The continuous line is the curve of the fast set, the broken line, the curve of the slow set. The ordinates are the averages; the abscissae, the three-day periods.

two three-day periods and also that there is a marked decrease in both the actual and percentage difference. During the last three of the three-day periods the difference was uniformly in favor of the sixty fast lines and was remarkably constant. Figure 16, the polygon of the average number of generations produced by each set of sixty lines per day during the consecutive three-day periods of this experiment, represents this same result graphically.

Figure 17-*a* and 17-*b* give the curves of variation from the diverse lines, the former for the first six days, the latter for the last nine days. Why the ex-conjugants of the fast selected lines should for the first five days be slower than the ex-conjugants of the slow selected ones is not clear. But this condition existed

only during the period of reorganization after conjugation, when the fission rate in all was extremely low. As soon as the 'normal' fission rate was resumed, the balanced-selected set of origi-

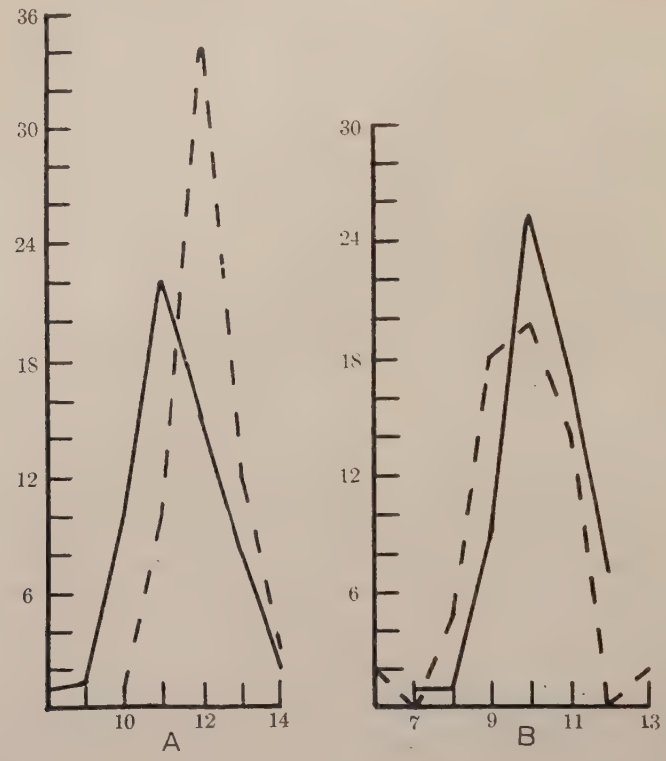

Fig. 17-*a* Curves of variation of the lines of Experiment 3-C (balanced selection after conjugation) for the first 6 days of the experiment. The continuous line is the curve of the fast set; the broken line, the curve of the slow set. The ordinates are the numbers of lines; the abscissae, the number of generations.

Fig. 17-*b* Curves of variation of the lines of Experiment 3-C (balanced selection after conjugation). The continuous line is the curve of the fast set; the broken line, the curve of the slow set. The ordinates are numbers of lines; the abscissae, generations.

nally fast ex-conjugants at once resumed its place in advance of the balanced-selected set of originally slow ex-conjugants and continued to divide more rapidly throughout the rest of the experiment.

Thus it is clear that the heritable difference in fission rate brought about by selection during vegetative reproduction is not lost when the animals conjugate, but persists through that ordeal.

Statement of the general results of the third series of experiments. This third series of experiments has entirely corroborated the results of the first and second series. In a second clone, entirely unrelated to that used for the first and second series of experiments, opposite selection for thirty days produced a heritable difference of average fission rate, a difference that gradually increased as selection progressed, indicating again that the effect of selection on this physiological character is cumulative. This average difference persisted through twenty-one days of balanced selection, twenty-nine days of mass culture followed by conjugation and then fifteen days of further balanced selection.

DISCUSSION AND CONCLUSIONS

All the experiments thus give concordant results; through selection of individual differences in fission rate it is possible to divide a clone into two divisions differing hereditarily in rate of multiplication. The effects of selection are cumulative; the hereditary differences between the two divisions become greater the longer selection continues. By reversing the direction of selection the hereditary differences between the sets are reversed.

Is this effect of selection due to the slow accumulation of small variations, or to the chance isolation of mutants differing markedly from the type? The whole character of the results indicates strongly that the former is the case, and this indication is borne out by careful study of the records. There is no sudden change at a definite point, indicating the appearance of a mutant. The steady cumulative effect of continued selection can not be explained on the mutant theory without giving such a meaning to the word mutant as removes any distinction between it and 'slight individual variation.' It would require us to assume the repeated appearance of successively faster and faster mutants in each of the thirty fast selected lines, of successively slower and slower mutants in each of the thirty slow-selected lines; a conception which coincides with the view that selection operates cumulatively on slight individual variations.

Woodruff and Erdmann ('14) show that in Paramecium during vegetative reproduction there are periodical reorganizations of the nucleus, the vegetative macronucleus being replaced by a new portion of the reserve micronucleus. It has been suggested that this new macronucleus may give an altered hereditary constitution, so that at each reorganization inherited variations may appear. Are the effects of selection herein described based on such variations occurring thus at the time of reorganization?

The relatively short time required for producing inherited differences among the progeny of a single individual make it improbable that the variations in Stylonychia are to be accounted for in this way. As we have seen, marked differences appear after ten days of selection, and these are gradually increased in the next ten days, and again in the next, and so on. The percentage of difference in proportion to the total average fission rate for the 13 consecutive ten-day periods of Experiment 1 are 5.41, 10.35, 5.58, 7.62, 23.81, 13.69, 19.20, 21.93, 15.19, 24.71, 26.05, 25.35 and 19.23. These percentages for the three consecutive ten-day periods of Experiment 3 are: 1.99, 4.36 and 7.10. Now in Paramecium the interval between reorganizations is about thirty days. If in Stylonychia the interval is of about this length, it would be quite impossible to account on this ground for the cumulative effects of selection occurring within periods much shorter; selection should show sudden effects immediately after the reorganization in a given stock, and should then be quite without effect during the intervening periods. Nothing of this sort appears in the records of the present experiments.

The main interest of this particular matter lies in its bearing on the question whether variations are definite and limited in extent and possible number, as in rigid Mendelian recombinations of invariable factors; or whether variations may be of indefinitely many diverse extents and are not limited by a precise numerically definable factorial structure of the germinal material. If the nuclear reorganization described by Woodruff and Erdmann takes place in a definite way, comparable to the known reductions and recombinations in the chromosomal apparatus at the formation of the germ cells, then this could not be

a source of indefinite and unlimited variation. After a time the possible combinations of factors would be exhausted, and such constancy would result as Johannsen claims to have found in his 'pure lines' of beans. Evolution thus could not make extended and continuous progress in this way. If on the other hand the nuclear reorganization occurs in no precisely definable way, but with indefinite and unlimited variations, then this apparatus shows precisely the characteristics held to be common to organisms by those who believe in continued evolutionary progress through the accumulation of such indefinite and unlimited variations. Some material basis for such variations would have to be assumed; the nucleus might furnish this as well as any other portion of the organism. But, as we have seen, the present evidence does not favor the idea that the hereditary variations in Stylonychia are dependent at all on these nuclear reorganizations.

Our main result, that during vegetative reproduction among the progeny of a single individual selection of small variations produces cumulative hereditary effects, is in marked contrast with the results of most investigators, who, following Johannsen ('03, '09, '11), have found that 'pure lines' or 'clones' are hereditarily constant under selection. Johannsen's results were obtained with self-fertilized lines of beans. Similar ineffectiveness of selection has been found by Hanel ('08) and Lashley ('15) as to the number of tentacles in Hydra multiply by budding, by Jennings ('08, '09, '10) for size in infusoria; by Barber ('07) (in the main), and by Winslow and Walker ('09), in bacteria; by East ('10) in the vegetative reproduction of the potato, by Agar ('13 and '14) in Cladocera and aphids multiplying parthenogenetically; and by various other investigators on diverse organisms. Some discordant results have been recorded, but most of these are ill-defined or uncertain; it is mainly in bacteria, with their immense difficulties for precise technique in pedigree work, that heritable variations or modifications have been described. The immense preponderance of evidence has been that in uniparental reproduction heritable variations do not occur (save as rare mutations of marked character), and that selection of slight

individual variations is without effect in altering the hereditary characteristics.

How are we to account for the discrepancy between the present results, and those just mentioned? In Stylonychia we are dealing with an organism which is large enough to be easily handled and followed individually, so that no question can arise as to the purity of the pedigrees (as sometimes occurs with reference to Bacteria). In this organism the facts as to the cumulative effect of selection are clear.

We are of course dealing with a delicate physiological characteristic, and this is perhaps more readily varied (even hereditarily) than the characters examined by most other investigators. Further, it is perhaps true that hereditary changes are more easily brought about in the Protozoa than in the more complex organisms, for in Protozoa the 'apparatus of heredity' is in close chemical contact with all the somatoplasm.

But a certain feature of the experimental procedure in the present case may have more importance than these conjectural considerations. It has been possible in my work to make a much greater number of actual selections (where plus and minus cases were both present to choose from), than in most of the work that has given negative results. And it has been found that few selections give very slight results, and that a great number are required to give any marked differences between the sets. Thus, in my main experiment, on the average 39.86 plus selections were made in the fast-selected lines; 34.36 minus selections in the slow-selected lines. The difference between the two sets was thus the equivalent of some 74 selections extending through an average of 150 generations. This resulted in the production of a constant average difference per line of 0.42 of one fission per day.

Contrast with this great number of selections the *six* made by Johannsen in obtaining his negative results with beans, the three or four made by East with potatoes, the two made by Winslow and Walker with bacteria, and similar small numbers made by most other investigators along these lines; even indeed the selection through fifteen generations made by Agar, in

Cladocera. It appears not at all inconceivable that in these organisms an equal number of selections, covering as great a number of generations as were made in Stylonychia, would have given similar heritable effects. What all the work shows (and here my own is not in positive disagreement) is that heritable variations of considerable extent do not occur so frequently as was at one time supposed, so that a few selections are not sufficient for establishing a definite positive effect. But negative results from a few selections are not sufficient for disproving the occurrence of heritable small variations which may be gradually accumulated. This indeed has been admitted by many of those that have obtained negative results; thus Johannsen remarked that "there is the possibility that a selection of fluctuating variants, during very many generations, might divert the type of a line" ('03, p. 62); Jennings says "what the pure line work shows (agreeing in this with other lines of evidence) is that the changes on which selection may act are few and far between instead of abundant . . ." ('10, p. 144), and East states that "as a result of these experiments I would not go so far as to say that variations in power of resisting physiological or fungus diseases do not occur in asexual reproduction, but I do believe that the relative possibility that the commercial grower will obtain disease-resisting varieties in this way is negligible" ('10, p. 134).

As a result of this work upon Stylonychia it is possible to substitute for such indefinite remarks, precise data as to the occurrence of heritable variations and their accumulation through selection, when sufficiently long continued. And this can hardly fail to have influence on the conception of the hereditary constitution or genotype as a fixed thing, changing only discontinuously by marked steps or mutations, that do not intergrade.

SUMMARY

In Stylonychia pustulata, by the opposite selection through more than 150 generations of small individual variations occurring among the progeny of a single individual, it was possible to produce two sets differing hereditarily in rate of fission. During selection there was a gradual increase in the average heritable

difference between the two sets, showing that the effect of selection was cumulative.

This result was, at various intervals, subjected to the most rigid tests possible, by balanced selection throughout long periods; by mass culture without selection, and by reversed selection. In every case the results were corroborated. The hereditary differences induced continued through periods of balanced selection lasting longer than the periods of direct selection by which they were induced; they did not disappear save under the effects of reversed selection.

These results were first reached with the progeny of a single individual multiplying asexually. They were then confirmed by beginning anew with a single individual from among this set and obtaining the same results among its progeny. A third set, derived from a wild individual quite unrelated to the first two series, gave the same results. In this third series conjugation occurred within each of the diverse sets produced through selection, and it was found that the hereditary differences persisted through and after conjugation.

Thus in Stylonychia, from a single clone of given genotype it is possible to obtain through long continued selection during reproduction by fission, two sets (clones?) of diverse genotype, differing characteristically from each other in rate of fission, under identical conditions; and retaining these differences from generation to generation. The selection of small variations, such as appear within the 'pure strain' or clone, is then an effective evolutionary procedure.

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